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Histone Deacetylase (HDAC) Expression Changes in the White Blood Cells of Sodium Valproate Treated Patients and Their Association with Obesity

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Abstract: **Background:** Sodium valproate (NaV) is a widely prescribed antiepileptic and mood-stabilizing drug that also functions as a histone deacetylase inhibitor (HDACi). HDAC deregulation contributes to cancer and neurodegenerative disorders, yet the feedback regulation of HDAC genes under pharmaceutical inhibition remains unclear. **Methods:** This pilot study evaluated mRNA expression of class I HDACs (HDAC1, HDAC2, HDAC3, HDAC8) and class IIa HDACs (HDAC4, HDAC5, HDAC7, HDAC9) using RT-qPCR in peripheral blood from 50 NaV-treated epileptic patients and 50 age/sex-matched neurological controls. **Results:** NaV treatment was associated with significant upregulation of HDAC1 (↑2.6-fold) and HDAC3 (↑2.1-fold), alongside downregulation of HDAC7 (↓1.9-fold). HDAC2 expression was unaffected by NaV but significantly reduced in smokers across groups. Obesity was linked to increased HDAC1 and reduced HDAC3 and HDAC9 expression. **Conclusions:** NaV therapy induces distinct de novo expression changes in HDAC genes, suggesting feedback regulation mechanisms. These findings provide a basis for larger studies examining HDAC superfamily expression as potential biomarkers of treatment response.

Key Words: Epigenetics, HDAC, Sodium Valproate, Qpcr, Gene Expression, Obesity

INTRODUCTION

Sodium valproate (NaV) is widely used to treat epilepsy and bipolar disorder. Beyond its neurological role, NaV acts as a histone deacetylase inhibitor (HDACi), implicating it in epigenetic regulation relevant to cancer, inflammation, and neurodegeneration [1]. While pharmacodynamic pathways of NaV have been studied extensively, the mechanisms underlying HDAC gene autoregulation and cross-regulation in humans remain poorly understood [2,3].

Given the potential for feedback loops in HDAC expression following NaV inhibition, this study aimed to investigate class I and IIa HDAC mRNA expression in peripheral blood of NaV-treated epileptic patients compared with neurological controls. We also evaluated the influence of smoking and obesity on HDAC expression [4,5].

Although the effects of NaV on epilepsy, bipolar disorder and migraine have been linked to several mechanisms, a precise route of action is still elusive. Certain mechanisms are supported by relevant research, including inhibition of succinic semialdehyde dehydrogenase [6,7], which leads to inhibition of GABA transaminase [8] and subsequently a GABA-mediated hyperpolarization and suppression of the postsynaptic neurons via the increasing influx of chloride ions [9]. In addition, NaV increases the biosynthesis of both GABA and its own receptors, inducing the neuronal response to GABA [7]. An antiepileptic property of NaV is its effect on fatty acid metabolism.

Reducing the incorporation of fatty acids into sterols and glycolipids improved fluidity and led to a higher threshold action potential thus lowering the response to abnormal excitatory signals [10].

NaV is currently also used in the management of various medical conditions including cardiovascular disease, renal disorders, endocrine disorders, muscular dystrophy, and neurodegenerative disorders [11,12]. Moreover, NaV was found to exhibit anti-microbial and anti-inflammatory activities as well as anti-cancer effects [13-15]. Both *in vitro* and animal studies have confirmed the synergistic effect of NaV in combination with arsenic trioxide in promoting cell cycle arrest and enhancing the apoptosis of lung cancer cells [16]. Likewise, a phase II clinical trial has shown that a combination regimen of NaV and CDK inhibitor (P276-00) delivered synergistic antiproliferative effects in non-small cell lung cancer, by upregulating tumour suppressor (p53, p21 and p27) and proapoptotic (Bax and Bcl-2) genes [17]. Multiple studies have provided evidence for the role of NaV in affecting the apoptosis proliferation and metastatic capacity in a number of *in vitro* models for different human cancers [15,18,19].

One of the most prominent characteristics of NaV, however, came with the discovery that it inhibits several histone deacetylases (HDAC) family members. Histone deacetylation is a well-known epigenetic modification causing silencing of the tumour suppressor genes in virtually all human cancers [20,21]. HDAC inhibition by compounds termed HDACis has been used in many clinical trials in combination therapeutic regimens of many cancer types, while a current clinical trial examines NaV treatment in the management of oral dysplasia [20,22]. The inhibitory effects of valproate on histone deacetylase (HDAC) have been demonstrated by multiple studies [23.24]. NaV treatment exerts transcriptome-wide epigenetic effects on rat cortical neurons, affecting gene expression responsible for neuronal excitation and inhibition [25]. The neuroprotective effects of NaV, are partially, at least, associated with a H3 hyperacetylation induced decrease in glyceraldehyde-3-phosphate dehydrogenase, which has proapoptotic activity [6]. Furthermore, it modulates DNA methylation status and alters the transcription factors expression, thus resulting in a chromatin remodelling [26-28].

NaV acts as HDACi class I on Histone H3 at lysine 9 and on Histone H4 at lysine 8, leading to gene expression changes of a large number of genes associated with the cell cycle and cell signalling [29,30]. Acetylation of Histone H3 and H4 in gene promoter areas is elevated in the neurons of animals treated with NaV [25]. Moreover, researchers have found that NaV and suberoylanilide hydroxamic acid (SAHA) can dysregulate stem cell differentiation and neurotrophic activity. That means it plays a role in the pathogenesis of neurodegenerative disease [31]. Various research studies have suggested that histone modification may serve as a marker of cancer progression [32,33]. The di- and trimethylated lysine 4 and mono- and demethylated lysine 9 of histone H3 (H3K4me2, H3K4me3, H3K9me, and H3K9me2) respectively, have been altered in various tumour types [33].

This study was designed to examine the expression of HDAC members of classes I and IIa, testing the hypothesis that inhibition of HDAC activity by NaV might lead to expression feedback loops. Thus, this study will compare the expression of these two HDAC class I and II members in the peripheral blood of epileptic patients treated with NaV and age/sex-matched control patients with other neurological conditions receiving different treatments.

Objectives

The study was designed with the following objectives:

- To compare mRNA expression of selected class I and IIa HDACs between NaV-treated epileptic patients and neurological controls
- To evaluate correlations between HDAC gene expressions
- To examine the influence of smoking status and BMI on HDAC expression
- To generate hypotheses for HDAC autoregulatory feedback under NaV exposure

METHODS

Samples and Data Collection

The study was conducted at the Al-Hada Armed Forces Hospital (Taif, Saudi Arabia), after receiving Ethics approval by the research ethics committee of the medical services' general directorate at the Armed Forces Hospital, Taif region, Saudi Arabia (Ref.No H-02-T-078). All research was performed in accordance with relevant guidelines/regulations. Written informed consent was obtained from all participants prior to their recruitment into the study. This observational study contained two groups: 50 epileptic patients receiving sodium valproate (NaV) as a standard treatment and 50 patients (age/sex-matched to the first group) with other neurological conditions that had no NaV in their treatment regimen. Patient characteristics, including age, gender, BMI, smoking, diagnosis and treatment regimen are provided in Table**1**. Seven ml of peripheral blood was collected in EDTA tubes (Thomas Scientific). Plasma and cellular components were separated by centrifugation and immediately stored at -80 °C.

RNA Extraction

Total RNA was extracted from the cellular component of the blood using the RNeasy® Mini kit (Qiagen, Germany) according to the manufacturer's protocol. Nanodrop (Themo Fisher Scientific, Inc.) was used to assess the quantity and purity of the extracted RNA.

Reverse Transcriptase cDNA

The high-capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific,) was used to synthesize cDNA, using the suppliers' protocol. The 20 µl cDNA product was diluted five times with distilled water. Quantitative PCR was conducted using 3 µl of cDNA (approximately 1/30 of the cDNA dilution) per reaction.

Quantitative Reverse Transcription PCR (QPCR)

The relative mRNA expression of HDAC family members (HDAC1, HDAC2, HDAC3, and HDAC8) and class IIa HDAC family members (HDAC4, HDAC5, HDAC7, and HDAC9) was assessed in this study. A competitive qPCR, utilising ACTB gene as endogenous control with a different fluorophore, was carried out using the Multiplex PCR Kit (QIAGEN, Germany) and a CFX96 Touch Real-Time PCR Detection System (BIO-RAD). Initial denaturation at 95°C for 2 minutes was followed by 40 cycles of denaturation at 95°C for 5 seconds and annealing at 60°C for 30 seconds during RT-qPCR. The sequences of primers and probes (Humanizing Genomics Macrogen, Korea) are provided in Table 2. The relative quantity (RQ) of each gene target's expression was calculated as RQ = $2^{(-\Delta\Delta Cq)}$, utilising the average DCq of the control group as biological calibrator for the DDCq calculation of each sample [34]. Triple technical replicates were conducted per sample and the average were used for subsequent calculations.

Statistical Analysis

Statistical analysis of the present study was performed with SPSS 24.0 software. The one sample Kolmogorov-Smirnov test indicated absence of normal distribution in the RQ values, therefore the non-parametric Mann–Whitney and Kruskal-Wallis tests were applied in comparisons of continuous variables in different categorical groups. Spearman's rank correlation was used to assess the degree of correlation between continuous values. The Bonferroni correction was used to adjust p values for multiple testing (8 independent targets).

RESULTS

Patient Characteristics

Statistical analysis demonstrated no significant differences in age, gender and smoking between the valproate and control groups. An association between BMI and valproate treatment was observed (Mann-Whitney test, p = 0.001), with higher BMIs observed in the valproate group (Table 1). Another trend appeared in relation to diabetes (p = 0.012) as all six diabetic patients were in the control group, but this is considered coincidental.

HDAC mRNA Expression

Relative mRNA quantification demonstrated in the valproate group a highly significant increase in mRNA expression for HDAC1 (Figure 1, Mann-Whitney test, adjusted p = 1.5×10^{-14}) and HDAC3 (adjusted p = 9.6×10^{-12}). In addition, a significant decrease in mRNA expression for HDAC7 was observed in the valproate group (Figure 1, Mann-Whitney test, adjusted p = 4.4×10^{-17}). No significant expression differences were observed in any of the other HDAC genes. A trend in HDAC8 proved insignificant upon Bonferroni adjustment. Interestingly, Spearman correlation analysis demonstrated moderate 2-tailed correlations between HDAC1 and HDAC3 (rho = 0.556) and HDAC1 and HDAC7 (rho = -574, indicating the inverse relationship). Not surprisingly, based on the above finding, HDAC3 was also inversely correlated with HDAC7 (rho = -622). No associations were found between any of the examined HDACs and age, gender, or any other medication except valproate.

Smoking and HDAC mRNA Expression

When testing the expression of the different HDACs in comparison to smoking status, only HDAC2 provided a significant difference, demonstrating a reduction of HDAC expression in smoking groups, both in the whole population (Mann-Whitney, adj. p = 8.9×10-8) and the separate valproate (adj. p = 6.0×10-6) and control (adj. p = 1.0×10-6) groups (Figure 2). In contrast, smoking did not affect mRNA expression in the remaining examined members of the family (HDAC1, HDAC3, HDAC8, HDAC4, HDAC5, HDAC7, and HDAC9).

Body mass Index and Class I and II HDAC Family mRNA Expression

Interesting associations were derived by examining HDAC expression among the different BMI groups. HDAC1 was higher in the obese group compared to normal and overweight patients (Kruskal-Wallis test, adj. p = 2.9×10^{-6}), while HDAC3 and HDAC9 were lower in the obese group (adj. p = 1.2×10^{-4} and adj. p = 3.2×10^{-5} respectively). Given the increase of HDAC1 and HDAC3 in the valproate group, we analyzed further these associations separately in the valproate and control groups (Figure 3). The differences in expression of HDAC1, HDAC3 and HDAC9 hold true when comparing the obese group against the combined normal and overweight groups in both control (Mann-Whitney test, adj p = 0.031, 0.028, 0.026 respectively) as well as in the NaV group (Mann-Whitney test, adj p = 0.001, 0.0006, 0.0005 respectively). These values must be treated cautiously though, as the total number of obese people in the sample is low (n = 9).

Table 1: Characteristics of the Patients Included in the Study

Parameters	NaV group	Control group	Difference
Total number	50	50	
Age, median (IQR)	37 (30-46)	36 (28-43)	NS
Gender			
Males	25	25	NS
Females	25	25	
Smoking			
Smokers	6	7	NS
Non-smokers	44	43	
BMI, median (IQR)	23.5 (20.7-26.3)	26.0 (24.0-29.0)	Mann-Whitney test, p = 0.001
BMI group			
Normal	15	30	X^2 test, p = 0.01
Overweight	29	17	
Obese	6	3	
Diabetic	0	6	X^2 test, p = 0.012
Non-diabetic	50	44	

NS: None-significant

Table 2: Primer/Probe Sequences Used for Rt-Qpcr Expression Analysis

Gene	F-primer	R-primer	Probe
HDAC1	CCAGATAACATGTCGGAGTACAG	AACTCAAACAGGCCATCGA	FAM-AGCAGATGCAGAGATTCAACGTTGGT
HDAC2	TCATAAGAAAGGAGCAAAGAAAG	GGTTGCTGAGCTGTTCTGAT	FAM-CACCACTGTTGTCCTTGGATTTATCTTC
HDAC3	GGGAAGTCTTGTTAGCTGCCTT	GATTGTCTGGCGGATCTGG	FAM-CAGATACTGGCGTGAGTTCTGATTCTCG
HDAC4	TGGGAAACGAGCTTGA TCCT	TGTGGAGGTTGTGCGCTG	FAM-AGGCAGCGCCAGTACTTGCTGTGG
HDAC5	ATCCAGAGTGCGTGAGGAC	CATGGCGCTCACAGTCTC	FAM-CCTCCTCGGTCTCACCTGCTTG
HDAC7	AACCTCAA TGCCA TCCGCT	GGTCACTGCCTCCACTTCTTCT	FAM-TCTGGAGGCCGTGATCCGGGT
HDAC8	AGTCCCGAGTATGTCAGTATGTG	CTTCAATCAAAGAATGCACCA	FAM-ACTCCCTGGCCAAGATCCCCA
HDAC9	AACTTGACACGGCAGCAC	CGATGCCTCTCTACTTCCTGT	FAM-CTCAGCTTCAGGAGCATATCAAGGAACTT
ACTB	CCAGCACAATGAAGATCAAGATCA	CATACTCCTGCTTGCTGATCCA	Cy5-CTCCTCCTGAGCGCAAGTACTCCGTG

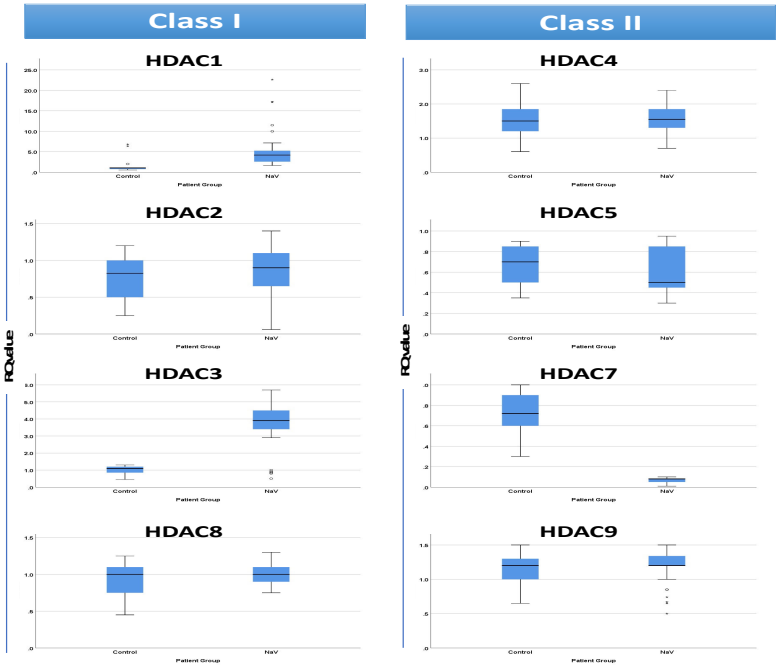

Figure 1: Boxplot Graphs Demonstrating the Range of MRNA Expression of Different HDACs between Patients Under Nav Treatment and Controls. HDAC1 And HDAC3 are Significantly Overexpressed While HDAC7 is Downregulated in the Nav Group

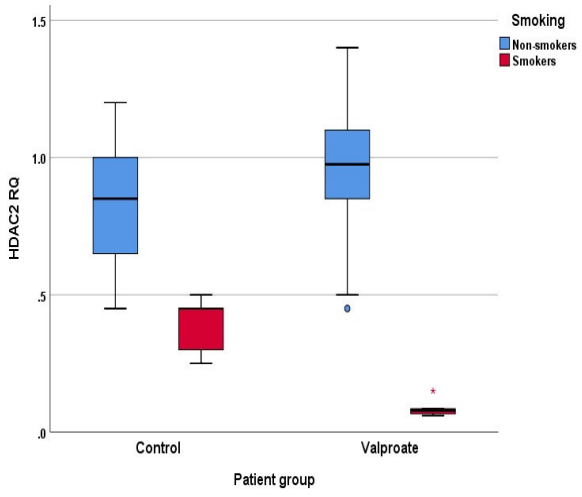

Figure 2: Clustered Boxplot Comparing the Mrna Expression of Hdac2 Gene by Smoking Stratum in the Valproate and Control Group. Hdac2 Expression Is Significantly Reduced in Smokers of Both Groups

DISCUSSION

The long-term effects of valproate extend beyond its direct involvement in modulating excitatory and inhibitory synaptic pathways and are related to the gene expression modulation [7]. In addition to its anticonvulsant activity, neuroscience benefits and ability to modulate neurogenesis, the epigenetic reprogramming that NaV exerts, plays a significant role in its complicated action [35]. NaV epigenetic effects are mainly mediated through the inhibition of histone deacetylase HDAC and the subsequent chromatin remodelling that induces multiple gene expression changes [36-38]. The effect of NaV on HDAC1 expression in epileptic patients has not been investigated to our knowledge, however, HDAC1 autoregulation has been described in previous *in vitro* studies. Mouse HDAC1 demonstrates an autoregulatory mechanism as HDAC1 protein is recruited to the *HDAC1* gene promoter by SP1 and NF-Y transcription factors and expression increases in the presence of trichostatin A (TSA, a known HDAC inhibitor) and co-expression of acetyltransferases [39]. Increased HDAC1 *de novo* expression in response to TSA treatment has been also shown in hepatocellular

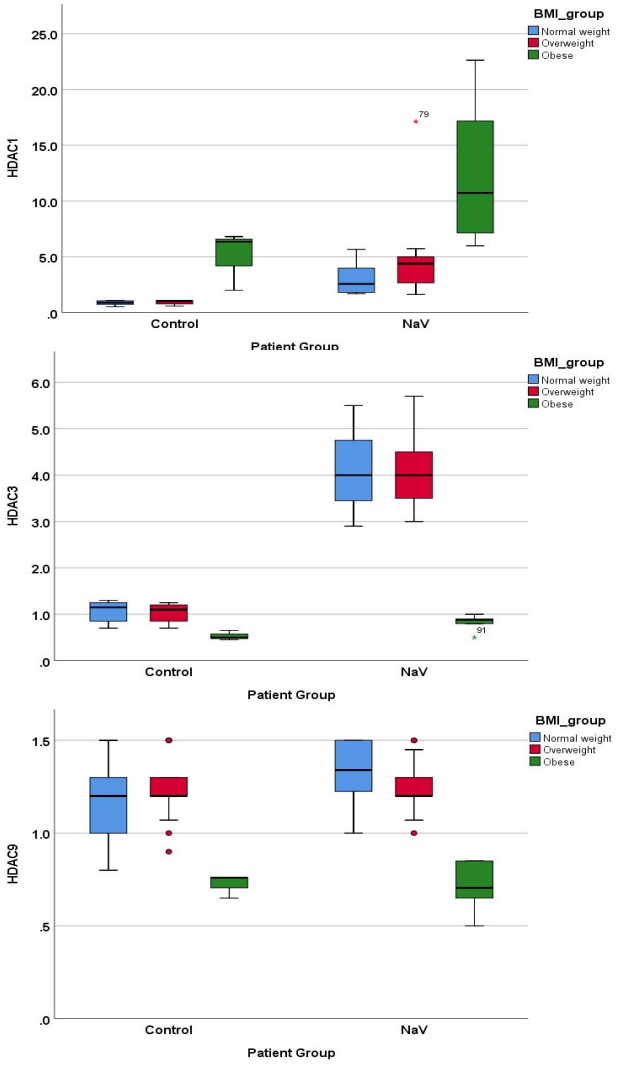

Figure 3: Clustered Boxplots Demonstrating Increased Expression of Hdac1 and Decreased Expression of HDAC3 and HDAC9 in the Obese Strata of Patients in both Nav Treatment and Control Groups

carcinoma cells and mouse fibroblasts, with the latter study, demonstrating further the dependence of HDAC expression to the presence of histone tail modification and chromatin organization [40,41]. Furthermore, a positive correlation was found between overexpression of HDAC1/2 and poor prognosis in gastric cancer GC patients. A study conducted by Jie Sun et al. demonstrated that NaV inhibited the activity of HDAC1/2 in GC cells, resulting in autophagy and apoptosis [42]. Induced apoptosis by NaV was caused by inhibition of the HDAC1/PTEN/Akt signalling pathway, as well as alterations to Bcl-2 and Beclin-1. A substantial proportion of the experimental results can be attributed to the high concentrations of NaV and its effectiveness. In contrast, NaV modulates the expression of a limited number of genes at standard therapeutic doses [43].

In contrast to our results, NaV was shown to downregulate HDAC3 expression in rat neurons, while HDAC1 and HDAC2 expression remained unaffected [44]. In addition, a study examining NaV-induced expression changes in the HDAC family in human ovarian cancer cells, showed that HDAC2 and HDAC7 genes were significantly downregulated after NaV treatment, at both mRNA and protein levels, while it did not affect any of the other HDACs [45]. The discrepancy between these studies and ours indicate that the effect of NaV on HDAC expression is dependent on the differentiation of the cells. This is not surprising as the epigenetic programmes of these cells differ significantly, and HDACs are modulators of this epigenetic programme. Therefore, this combined data demonstrate that NaV-based inhibition of HDAC activity leads to diverse feedback loops affecting de novo expression of HDAC members and supporting the need for further research to elucidate the molecular factors (transcription factors, other epigenetic modifiers, etc) that contribute into these feedback loops. Due to the observational nature of this study, we cannot exclude the possibility that the expression differences seen in the patients' blood for HDAC1, HDAC3 and HDAC7 may be related to the disease (i.e. epilepsy) *per se* rather than the treatment. This would require measuring expression in the blood of epileptic patients prior to the start of their treatment, which becomes challenging. However, there is adequate *in vitro* evidence showing such expression difference as a result of NaV treatment, but additional experimental evidence is required to exclude the disease-causing hypothesis.

While HDAC2 expression was not affected by NaV in our sample set, both the NaV and control groups, demonstrated a significant reduction in HDAC2 expression among smokers. This has been already reported in previous studies. HDAC2 expression reduction has been observed in the alveolar macrophages in smokers [46,47]. The mechanism is still not well understood, however, evidence suggests that smoking-induced reduction in HDAC2 expression is associated with enhanced phosphorylation of Akt in the PI3K-δ/Akt signalling pathway both in mouse asthma models and human asthma pediatric patients [46.48]. The patients treated with NaV in our study, demonstrate higher BMIs in comparison to the control group. This has been previously reported in the relevant literature as a known side effect of long-term NaV treatment [49-53]. NaV-induced obesity may be associated with the development of metabolic syndrome, while hyperinsulinemia and elevated serum triglyceride levels have been observed in valproate-treated patients, however the detailed mechanism behind this is not understood [50,54]. Our results indicate that obesity is associated with higher levels of HDAC1 and lower levels of HDAC3 and HDAC9, even when the analysis is stratified by the treatment group. While HDAC changes are expected to cause a degree of epigenetic reprogramming and highly likely affect metabolism, our observation of obesity needs to be treated with caution as there is only a limited number of obese patients in this study. Interestingly, HDAC1, HDAC3 and HDAC9 expression were lower in the visceral adipose tissue and subcutaneous adipose tissue of obese females, compared to females with normal weight, demonstrating

only partial agreement [55]. It is an important thought to stress again that our study examined white blood cells instead of adipose tissue.

CONCLUSION

This pilot study demonstrates that sodium valproate modifies HDAC gene expression in human blood, notably increasing HDAC1/3 and decreasing HDAC7. Smoking suppresses HDAC2, while obesity alters HDAC1/3/9 patterns. These findings support HDAC feedback regulation under NaV and provide rationale for larger, longitudinal, protein-level studies.

Strengths and Weaknesses

- **Strengths:** First in vivo human HDAC-NaV feedback data; matched controls; robust statistics; ethical rigor.
- **Weaknesses:** Observational design; no protein-level validation; lack of NaV dose-response analysis; small subgroups

Implications for Practice

- Epigenetic variability under NaV suggests personalization of therapy
- Smoking-related HDAC2 suppression may affect drug response
- BMI-related HDAC changes may underlie NaV-associated weight gain
- Potential for predictive biomarkers, but not yet clinically actionable

Limitations

- Cannot exclude epilepsy-related effects
- No protein-level validation
- No pre/post treatment analysis
- Obese subgroup small (n = 9)
- Pilot size limits generalizability

Future Directions

- Expand to full HDAC superfamily
- Validate with protein/chromatin assays
- Longitudinal pre/post NaV studies
- Multicenter, larger cohorts
- Investigate predictive biomarker potential

Ethics Approval and Consent to Participate

Approval for this study was sought and obtained by the research ethics committee of the medical services' general directorate at the Armed Forces Hospital, Taif region, Saudi Arabia (Ref.No H-02-T-078). All research was performed in accordance with relevant guidelines/regulations. Written informed consent was obtained from all participants prior to their recruitment into the study. Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

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